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TRANSMITTAL OF APPEAL BRIEF (Large Entity)	Docket No. 05090001BA

In Re Application Of: K. Okada, et al.

OIPE JC174 MAY 3 1 2005 PATENT & TRADEMARK OFFICE

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
10/035,156	January 4, 2002	B. T. L. Nguyen	30743	1641	2583

Invention: **IMMUNOASSAY METHOD AND IMMUNOASSAY KIT**

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on **April 18, 2005**

The fee for filing this Appeal Brief is: **$500.00**

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re patent application of Okada et al. — Group Art Unit 1641
Serial No. 10/035,156 — Examiner Nguyen
Filed January 4, 2002 — Confirmation No. 2583

For: ***IMMUNOASSAY METHOD AND IMMUNOASSAY KIT***

MAIL STOP APPEAL BRIEF
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPELLANTS' BRIEF UNDER 37 C.F.R. § 41.37

In response to the action of the Primary Examiner in finally rejecting claims 1-7 of this application, a Notice of Appeal was timely filed April18, 2005. This brief, which is filed herewith in triplicate, is in furtherance of the Notice of Appeal.

This brief contains these items under the following headings and in the order set forth below, as required under 37 C.F.R. § 41.37:

I. REAL PARTY IN INTEREST
II. RELATED APPEALS AND INTERFERENCES
III. STATUS OF CLAIMS
IV. STATUS OF AMENDMENTS
V. SUMMARY OF CLAIMED SUBJECT MATTER
VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL
VII. ARGUMENTS
- ☐ ARGUMENT VIIA. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

I. REAL PARTY IN INTEREST

The real party in interest in the appeal is:

- ☐ the party named in the caption of this brief.
- ☑ the following party:

  Netto Denko Corporation of Osaka, Japan.

II. RELATED APPEALS AND INTERFERENCES

With respect to other appeals or interferences that will directly affect, or be directly affected by, or have a bearing on the Board's decision in this appeal:

☑ there are no such appeals or interferences.

☐ these are as follows:

III. STATUS OF CLAIMS

The status of the claims in this application is as follows:

A. Total number of claims in Application

The claims in the application are: Claims 1-7, totaling 7 claims

B. Status of all the claims:

1. Claims cancelled: None
2. Claims withdrawn from consideration but not cancelled: None
3. Claims pending: Claims 1-7
4. Claims allowed: None
5. Claims rejected: Claims 1-7
6. Claims objected to: None

C. Claims on Appeal.

The claims on appeal are: Claims 1-7

IV. Status of Amendments

The status of amendments filed subsequent to the final rejection is as follows: There are no after-final amendments.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The claimed invention, defined in independent claims 1, 6 and 7, and in dependent claims 2-5, is directed to the detection of at least two different test substances (e.g. verotoxin producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; and verotoxin producing *Escherichia coli* and human hemoglobin) in a single test using the same base material, i.e. on a single test strip (see Technical Field of the Iinvention on page 1; first paragraph of the Summary of the Invention on page 3; and the last paragraph of page 21). The present invention thus allows the detection of diverse but related materials, detecting two of: the causative agent of a disease (*Escherichia coli* bacteria), the agent produced by the bacterium that is the immediate cause of disease (verotoxin); and the physiological effect that is produced (hemoglobin, e.g. in feces as a result of internal hemorrhage). This aspect of the invention is disclosed at least on page 2 of the application, where it is noted that the assay kit will detect "...verotoxin and human hemoglobin associated with internal hemorrhage...".

Prior to the claimed invention, <u>separate</u> tests for the detection of verotoxin and the bacteria that produce verotoxin (*Escherichia coli*) were employed. The tests were typically conducted in a time-consuming and labor intensive manner. For example, the bacteria were detected by culturing a test sample thought to harbor the bacteria, and then detecting bacterial antigens (e.g. O157) using an enzyme-linked immunosorbent assay (ELISA) method, or by latex agglutination. In order to detect the verotoxin that is produced by the bacterium, a different test was used, which also involved culture of a sample and detection of verotoxin 1 and/or verotoxin 2 by latex agglutination. These methods detected *E. coli* antigen and verotoxin in single, separate tests, and both required culturing of the sample prior to detection. The present invention provides a streamlined, efficient immunity chromatography method to permit rapid and highly accurate detection of the two target substances in a single test.

The method involves the simultaneous detection of at least two target assay substances. The target substances may be located in a liquid test sample, and detection of the target substances occurs during movement of the liquid test sample up a test strip

(immunity chromatography, see page 3, lines 3–4). Each target assay substance is detected by binding to two different immunity substances, e.g. antibodies, both of which are specific for the target substance (page 5, lines 25-26). One of the antibodies (the "first immunity substance") unlabeled and is immobilized on the test strip; the other (the "second immunity substance") is labeled and is not immobilized, but is present either 1) in a liquid (see page 9, lines 16-17), or 2) temporarily dried onto the test strip (see page 10, lines 2-5).

If in a liquid, the second immunity substance may be in the liquid test sample, where it binds to the target substance to form a complex. The complex then migrates up the test strip, encounters the immobilized first immunity substance, and is sequestered there. Alternatively, the second immunity substance may be in a liquid separate form the test sample. In this case, the test sample liquid containing the target substance first moves up the strip, where the target substance binds to and is sequestered by the first immunity substance. Then, a liquid containing the labeled second immunity substance moves up the strip, allowing the second immunity substance to bind to the sequestered target substance. A third variation is that the test sample itself may be dried onto the test strip (below the immobilized first immunity substance). The liquid containing the second immunity substance is allowed to move up the strip, the target substance is dissolved by the liquid and binds to the second immunity substance, and the complex continues to move up the strip to encounter the immobilized first immunity substance, where it is sequestered by specific binding between the target substance (in the complex) and the immobilized first immunity substance.

On the other hand, if the second immunity substance is dried onto the test strip, it is located at a position near the end that will be immersed in (or other wise receive) the test sample. In this case, the liquid containing the target substance migrates up the test strip, and the liquid dissolves the second immunity substance, which then binds to the target substance, forming a complex. The complex then migrates up the strip and encounters the immobilized first immunity substance, where it is sequestered by specific binding between the target substance (in the complex) and the immobilized first

immunity substance. According to the invention, two target substances in a single test sample may be detected in this manner on a single strip by using two different labeled second immunity substances, and by immobilizing two different first immunity substances at different positions on a single test strip.

Both of the two antibodies for a single target substance may be polyclonal antibodies, or one may be monoclonal and the other polyclonal, or both may be monoclonal, so long as both monoclonals recognize and bind to different epitopes of the target substance (page 6, lines 4-7).

Specifically, independent claim 1 is directed to an immunoassay method for detecting, in a single assay, <u>two</u> target substances selected from one of three possible combinations of two substances:

1) verotoxin producing *Escherichia coli* and verotoxin;

2) verotoxin and human hemoglobin; and

3) verotoxin producing *Escherichia coli* and human hemoglobin.

Independent claim 6 is directed to an immunoassay device for carrying out the method, and independent claim 7 is directed to an immunoassay kit for carrying out the method.

The method of claim 1 comprises two steps:

Step 1 involves: bringing an immobilized phase comprising (at different positions on a water-absorbable base material) at least two different first immunity substances that are <u>specific for assay target substances in a test sample</u> into contact with a test sample and a liquid containing second immunity substances. Each of the second immunity substances is labeled with colored latex particles, and is capable of binding specifically one of the target substances, thereby forming a labeled target substance-second immunity substance complex. The complexes also bind with respective first immunity substances at the immobilized phase, i.e. they are sequestered at the immobilized phase, by the <u>specific binding between a first immunity substance and the target substance of the complex</u>. The binding of a target substance to the first and second immunity substances may occur in any order, i.e. the target substance may first bind a labeled second immunity substance to form a complex, and the complex then binds to an immobilized first immunity substance

specific for the target substance. Alternatively, the target substance may first bind to the immobilized first immunity substance, and the labeled second immunity substance may then bind to the target substance that is bound (immobilized) with the first immunity substance.

The second step of the method involves: detecting the labeled immunity substance complex (the label being derived from the second immunity substance).

Thus, in all embodiments of the invention, target substances (if present) are located in the test sample, and will eventually be immobilized on the strip by first immobilized immunity substances that are specific for each of them. An immobilized target substance bound to the first immunity substance would not be readily detectable. Detection of the bound target substance is made possible by the binding of the labeled second immunity substance.

The dependent claims recite features of the invention with respect to the order in which the reactive substances make contact with one another, as follows:

Claim 2: Contact is made by flowing the test sample so that it is absorbed from one end of the water-absorbable base material, thereby to bind the complex with the first immunity substance; (i.e. the complex between the target substance and the second labeled immunity substance is made first, in the liquid test sample itself; then the liquid containing the complex flows up the strip to bind and be sequestered by the first immunity substance, which is immobilized on the strip; see also the fourth paragraph of page 11, and Figures 1 and 2);

Claim 3: Contact is made by flowing the test sample so that it is absorbed from one end of the water-absorbable base material, thereby to bind the assay target substance with the first immunity substance, and then flowing the liquid to allow absorption thereof by the base material, thereby to bind the second immunity substance with the assay target substance (i.e. two steps are carried out: first, the liquid sample containing the target substance flows up the strip and binds and is sequestered by the first immunity substance; second, a liquid with the labeled second immunity substance flows up the strip and binds to the test substance that is bound to the first immunity substance on the strip, thereby

labeling the test substance, see also last paragraph of page 11, which continues onto page 12, and Figures 1 and 2).

Claim 4: Contact is made by absorbing the test sample to the strip halfway up to the immobilized phase, and allowing the liquid containing the second immunity substance to be absorbed from one end of the water-soluble base material and flow up the strip, thus encountering the test substance and forming a complex between the test substance and the second immunity substance , followed by binding the complex with the first immunity substance at the immobilized phase (i.e. sample is absorbed onto the strip in the middle, a liquid containing the second immunity substance flows up the strip and the second immunity substance binds the test substance, forming a complex, then the complex continues to travel up the strip and binds the first immunity substance at the immobilized phase; see also the first complete paragraph of page 12).

Claim 5: wherein contact is made by positioning the second immunity substance half way up the strip and drying the strip; the test sample containing the test substance is then absorbed from one end of the strip forming a complex with the second immunity substance , followed by binding of the complex to the immobilized first immunity substance (i.e. second immunity substance, so that a mobile test substance -second immunity substance complex is formed. The complex continues to flow up the strip until it encounters the first immunity substance, whereupon the test substance is bound to and sequestered by the first immunity substance; see also paragraph number 6 on page 4 and Figures 3 and 4).

In summary, as recited in claims 1, 6, and 7, the present invention provides a method, device and kit (respectively) for detecting on a single test strip, two substances:

1) verotoxin producing *Escherichia coli* and verotoxin;

2) verotoxin and human hemoglobin; or

3) verotoxin producing *Escherichia coli* and human hemoglobin.

Prior to the present invention, no single test was available for the simultaneous detection of any two of these entities.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The sole issue presented in this Appeal is whether Claims 1-7 are obvious over a combination of U.S. Patent 5,965,458 to Kouvonen et al. ("Kouvonen") in view of U.S. patent 6,080,400 to Williams et al. ("Williams") and U.S. patent 5,512,282 to Krivan et al. ("Krivan").

ARGUMENT VIIA. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

There are no rejections under 35 U.S.C. §112, first paragraph.

ARGUMENT VIIB. REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

There are no rejections under 35 U.S.C. §112, second paragraph.

ARGUMENT VIIC. REJECTIONS UNDER 35 U.S.C. §102

There are no rejections under 35 U.S.C. §102.

ARGUMENT VIID. REJECTIONS UNDER 35 U.S.C. §103

The Prior Art

Pursuant to an Office Action dated January 24, 2005, (the "Final Rejection"), claims 1-7 were erroneously rejected under 35 U.S.C. §103 as unpatentable over a combination of U.S. Patent 5,965,458 to Kouvonen et al. ("Kouvonen") in view of U.S. patent 6,080,400 to Williams et al. ("Williams") and U.S. patent 5,512,282 to Krivan et al. ("Krivan"). Applicants respectfully submit that claims 1-7 are not obvious over the combination put forth by the Examiner, since, among other considerations, none of the references shows or suggests an assay for simultaneously detecting two substances from the following group of three possible combinations of two substances: verotoxin producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; or verotoxin producing *Escherichia coli* and human hemoglobin.

Kouvonen (U.S. patent 5,965,458)

The focus of Kouvonen is the development of an improved test strip for rapid immunoassays. Immunoassay strips with immobilized antibodies were well known prior to Kouvonen and many are described beginning in column 2 at line13 and ending column 3 at line 11. The rationale for developing yet another test strip is given in column 3 at lines 12-22, which describes the need for a test strip that is very simple, and that can be used almost anywhere (even without electricity or clean water) and even by untrained personnel. The test strip should be "rapid, sensitive and reliable" (column 3, lines 18-19), and construction of the strip should be simple enough to "allow manufacturing without complicated methods" (column 3, lines 19-21). Nevertheless, the product should be strong enough to "withstand environmental field conditions" (column 3, line 22).

The entire focus of Kouvonen is the design of a test strip that meets these criteria. The initial description of the innovative strip in the patent states that the structure is "simple and advantageous in view of manufacture"(column 3, lines 24-25 and 28-30). The use of the strip involves simply dipping the test strip into a sample and observing the results (column 3, lines 25-26), however the unique, distinguishing feature of the test strip is that "the essential reactions take place at a protected chamber-like gap" (column 3,

lines 27-28). This feature is spelled out in great detail in the specification, and is clearly illustrated in Figures 1-3. For example, in column 3 at lines 44-48, the following description is given: "Said test strip is characterized in that said backing and said membrane limit between themselves an *air gap which it open at its edges*. Said chamber will function as a *sheltered reaction chamber* for the immunological reaction taking place in said membrane." (Emphasis added.)

A more detailed description of the strip, and particularly of the air gap/sheltered reaction chamber is given in conjunction with the description of the figures, a copy of which is presented in Evidence Appendix IX. Indeed the design is relatively simple, involving, with reference to exploded Figure 1B, backing sheet 1 and cover 6, which enclose absorbing pads 3 and 5, with test membrane 2 between and immediately adjacent to cover 6 (in Figure 1B) or to backing sheet 1 (Figure 3). In either case, absorbing pads 3 and 5 are "separated by gap 4 which is long enough to extend over the reaction areas in test membrane 2" (column 4, lines 61-62). Gap 4 is clearly depicted in Figures 1A, 1B and 3, and is the distinctive air gap/sheltered reaction chamber of the test strip of the invention. The reactive species of the test strip include zone 7 on pad 3, which contains a dried labeled reagent, and at least one antibody line 8 (and possibly control line(s) 9) on membrane 2. During use of the strip, a test sample moves by lateral flow (column 5, line 37) up the strip and any analytes in the sample encounter the dried labeled reagent of zone 7 and the antibody of line 8. A key feature of the invention is that, during the flow of the sample, the gap 4 between test membrane 2 and backing 1 provides a small chamber, open on both sides, where the liquid flow on membrane 2 is undisturbed, i.e. the air gap/sheltered reaction chamber. Further, since gap 4 is narrow, the atmosphere in the gap retains its humidity and any drying caused by air will not disturb the flow of liquid (column 5, lines 6-13).

These features of the invention are reflected in the claims of Kouvonen. Claim 1 recites that the cover of the test strip is positioned over the supporting pads in a spaced apart relationship to define "top and bottom boundaries of an air gap" which is "open at its edges".

Applicant notes that the problem solved by the invention of Kouvonen is the previous lack of simple, reliable test strips that are easy to manufacture. The solution to the problem, according to Kouvonen, is the test strip with an air gap/sheltered reaction chamber. The discussion of what or how many substances could be detected with the strip is confined to general "laundry lists" of potential substances of interest, for example in columns 8 and 9, where many substances are listed. Applicant submits that in these lists, there is no suggestion of particular substances that are well suited for detection by the strip, and absolutely no suggestion or discussion of the benefits of pairing the detection of any two particular substances. The detection of hemoglobin is mentioned (column 8, line 30); the detection of microorganisms is mentioned (column 9, line 25). However, there is neither a showing or suggestion of the detection on a single strip, or advantages thereof, of the combinations of substances recited in the claims of the present invention (verotoxin producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; and verotoxin producing *Escherichia coli* and human hemoglobin). Rather, the invention of Kouvonen involves the <u>design</u> of the test strip itself.

Applicant further notes that the design requirements of the strip of Kouvonen differ from those of the present invention. In all embodiments of the strip of Kouvonen, zone 7 in absorbing pad 3 contains a dried labeled reagent, e.g. latex, metal colloid or other particles as well as soluble molecules (column 4, lines 56-57; column 5, lines 43-44), and antibodies are immobilized at antibody lines 8 (column 4, lines 64-65). Further, claim 1 of Kouvonen recites "at least one label zone downstream of said sample absorbing end, and at least one immunochemical reagent zone located downstream of said label zone". This is in contrast to the present invention in which <u>the label is attached to the second immunity substance</u>, which may be either in a mobile liquid phase (claims 2-4) or dried onto the strip (claim 5).

Further, the method of the present invention need not be carried out on a strip per se, but may be carried out using a water-absorbable base material of any shape, so long as the assay target substance can be developed thereon (page 7, third full paragraph). For example, the method could utilize a rod shaped base material. In contrast, claim 1 of

Kouvenon recites a "test strip".

While Kouvenon broadly recites that different analytes may be detected on the test strip disclosed therein, there is absolutely no disclosure or suggestion therein of detecting two different kinds of assay target substances as is done in the method of the present invention. At most, Kouvenon teaches detecting two types of similar analytes. For example, Example 3 of Kouvenon describes a test strip designed to determine the presence of human blood by detecting human hemoglobin and human albumin, both of which are proteins found in the blood. In contrast, the present invention allows the detection of diverse materials such as verotoxin and the bacteria that produce it, allowing the detection of two of: the causative agent of a disease (the bacteria), the agent it produces to cause disease symptoms (verotoxin), and the physiological effect that is produced (hemoglobin). No such detection system is shown or suggested by Kouvenon.

Williams (U.S. patent 6,080,400)

The primary focus of Williams is the provision of antitoxin therapy to neutralize the pathological effects of verotoxins produced by *Escherichia coli*. The background section of the application describes in detail the untoward effects of verotoxins (see section C, beginning in column 4 at line 13), and also describes the lack of available treatments (see section E, beginning in column 9 at line 34). The last sentence of section E (column 10, lines 13-15) states that "What is needed is a means to block the progression of disease, without the complications associated with antimicrobial treatment." The invention supplies such a means, as stated in column 16 at lines 62-63: "The present invention relates to antitoxin therapy for humans and other animals." In addition, the use of antitoxin for diagnostic purposes, e.g. to detect the presence of toxins in samples, is also disclosed (column 18, lines 12-13; column 21, lines 8-10).

Antibodies to *E. coli* verotoxins were known prior to the invention of Williams. Some examples are described in section A (column 24 beginning at line 42). Thus, the ability of make antibodies to verotoxin is not the point of the Williams invention. Rather, a key feature is the *method* of producing the antibodies. Problems with prior art approaches include suboptimal recovery of the protein to be used to elicit antibody

production (column 22, lines 1-8 and 15-25), as well as problems with production of neutralizing antibodies, for which "success has not been consistently achieved" (column 24, lines 49-50).

The Williams invention addresses these problems by providing methods of recovering relatively high levels of protein in which the conformation of the protein is correct, i.e. the protein is in its *native conformation* (see column 22, lines26-43), and to produce superior, improved antitoxin to the native form of the protein *in avian species* (see column 26, lines 4-6 and 14-15).

Williams states that antitoxin produced in this manner can be used therapeutically or for diagnostic purposes. Williams discussion of the diagnostic use of the antibodies is given briefly in column 18, at lines 12-36, and in detail in column 30 in section VII, which begins at line 21. The statement is made at column 30, lines 22-23 that "The invention contemplates detecting bacterial toxin in a sample." Section VII goes on to describe the types of samples that can be analyzed (column 30, lines 28-49); the steps of carrying out a competitive immunoassay (column 30, lines 50-67 and column 31, lines 1-3); the steps of carrying out a "sandwich" assay (column 31, lines 4-20); and other various embodiments (e.g. pouring sample liquids over antibody immobilized on a support). Applicant notes that all the methodology that is discussed by Williams is known methodology; the inventive feature of Williams is not the development of a new method of carrying out a diagnostic test, but rather is the improved method of obtaining the improved antibodies that are used in the test.

Applicant notes that Williams does not at any point in the patent show or suggest the detection of verotoxin together with another substance on the same test strip, as is required in claims 1, 6, and 7 of the present application. Further, Williams does not at any point show or suggest the detection of *E. coli* bacteria by any method whatsoever. The Williams technology is focused on novel methods of producing improved antibodies for use in the treatment of verotoxin related illnesses, and the use of those same antibodies for the detection of verotoxin (and related toxins) in samples. There is no showing or suggestion that the detection of verotoxin could or should be coupled with the detection

of *E. coli* on a single test strip, as is the case for the present invention, nor are the need for or advantages of such a detection system discussed.

Krivan (U.S. patent 5, 512, 282)

The main focus of Krivan is the provision of antibodies to "Shiga-like" toxins or "SLTs" (an alternative nomenclature for verotoxins). At the time of filing of the Krivan application, which was four years prior to Williams, the only method to detect the toxins was a laborious and time-consuming procedure involving determination of cytotoxicity to cells in culture. Not only did the procedure require a lengthy period of time before results could be received, a bona fide cell culture facility was required to carry out the assay (see the Abstract, and column 2, lines 51-62). Krivan states that the point of the invention is that "We have discovered that pregnant cows immunized with purified SLT's produce monospecific, polyclonal antibodies to SLT's that are of a surprisingly and unexpectedly high titer. As a result, we were able to produce very high titer colostrum and milk for use in passive immunization or treatment of SLT toxin." (Column 5, lines 42-47). The text further states that, in contrast to earlier methods, the cows did not experience ill effects as a result of the immunization, so that active toxin could be used for the immunization. As a result, the antibodies that were produced recognized native epitopes and displayed high valency, so that "...as a result of this increased polyvalency, we were able to produce purified IgG that provides outstanding signal to noise ratio when used as reagent in assays for the detection of SLTs." (Column 5, lines 47-56).

Uses of the antibodies described by Krivan include therapeutic, diagnostic, and scientific applications (column 10, lines 28-30). The diagnostic application is discussed in detail beginning in column 11 at lines 38-40, which state that "The IgG and antibodies of the invention are also useful for detecting the presence of one or more SLTs or SLT-producing bacteria in a sample suspected of containing such toxins or bacteria." Applicant notes that the detection of SLT-producing bacteria referred to in this passage must, however, be indirect, i.e. what is actually detected is the toxin (not the bacteria) and the presence of the bacteria can be inferred only from a positive toxin result. (This assumes that typically the only source of the toxin would be a bacteria, and excludes the

possibility of, for example, detecting the direct intake of the toxin itself.) This is because the antibodies produced by the methods of Krivan are not specific for the bacteria, but for the toxin produced by the bacteria. Krivan does not disclose or discuss antibodies that are specific for SLT-producing bacteria, but only for the toxin molecule produced thereby. In fact, Krivan teaches away from detecting the bacteria at all, stating in column 14 at lines23-26: "Because there are over 50 serotypes of verotoxin-producing *E. coli*, any satisfactory overall diagnostic strategy must be directed toward detecting the verotoxin *rather than the organism*." (Emphasis added.)

Krivan's discussion of detection methods involves several "laundry lists" of potential variations, none of which are meant to convey novelty to the method. Clearly, the point of the invention is not whether the antibodies are immobilized on a particular type of surface (see column 11, lines 53-55 and column 12, lines 1-9), or which type of detection method is used (see column 12, lines 43-52). Rather, the focus of the Krivan invention is on the provision of particularly effective polyvalent antibodies that are specific for Shiga-like toxin molecules.

Applicant submits that a combination of Kouvonen, Williams and Krivan could at best provide a system for detecting the presence of verotoxin in a sample, possibly together with some similar substance. In particular, in view of the teaching of Krivan that the detection of bacteria should not be attempted due to the large number of existing serotypes, such a combination would not include *Escherichia coli*. The system would involve the use of the test strip developed by Kouvonen, and antibodies specific for verotoxin produced either by the improved methods of Williams, or the improved methods of Krivan (or both?). In any case, there would be no reason based on the teachings of these three references, to include detection of the groupings of substances recited in independent claims 1, 6, and 7 of the present invention (verotoxin producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; and verotoxin producing *Escherichia coli* and human hemoglobin) since none of the three references alludes to these combinations, or to any advantages that the detection of such combinations in a single test might afford.

In contrast, detection of two or more substances in the same assay or kit is a key feature of the present invention that is not taught or contemplated by the prior art. This process allows the rapid and simple evaluation of both the kind of infection and the extent of pathology associated with the infection. As described above, it is noted on page 2 of the application that "...verotoxin and human hemoglobin associated with internal hemorrhage..." (emphasis added) may be detected simultaneously by the kit of the present invention. Further, Examples 1-16 of the application represent the results of the detection of *E. coli* and/or verotoxin, and/or hemoglobin, which may be present without concurrent infection as in sample 15. Clearly, such an assay method is not rendered obvious by any combination of the cited references.

ARGUMENT VIIE. REJECTION OTHER THAN 35 U.S.C. §§102, 103 AND 112

There are no rejections other than under 35 U.S.C. §§ 102, 103, and 112.

VIII. Claims Appendix

The text of the claims involved in this Appeal are:

1. An immunoassay method comprising:

bringing an immobilized phase comprising, at different positions on a water-absorbable base material, at least two different first immunity substances wherein said first immunity substances are specific for assay target substances contained in a test sample that are selected from the group consisting of a combination of verotoxin-producing *Escherichia coli* and verotoxin, a combination of verotoxin and human hemoglobin, and a combination of verotoxin-producing *Escherichia coli* and human hemoglobin, into contact with a test sample and a liquid containing second immunity substances, wherein each of said second immunity substances is labeled with colored latex particles and binds with said assay target substances, thereby to form assay target substance-labeled immunity substance complexes and to bind said complexes with respective first immunity substances at the immobilized phase; and

detecting said labeled immunity substance complex.

2. The immunoassay method of claim 1, wherein the contact is made by flowing the test sample, so that it is absorbed from one end of the water-absorbable base material, thereby to bind said complex with the first immunity substance.

3. The immunoassay method of claim 1, wherein the contact is made by flowing the test sample, so that it is absorbed from one end of the water-absorbable base material, thereby to bind the assay target substance with the first immunity substance, and then flowing the liquid to allow absorption thereof by the base material, thereby to bind said second immunity substance with the assay target substance.

4. The immunoassay method of claim 1, wherein the contact is made by having the test

sample absorbed halfway up to the immobilized phase, allowing the liquid to be absorbed from one end of the water-absorbable base material, thereby to form a complex of said second immunity substance and the assay target substance, and binding said complex with the first immunity substance at the immobilized phase.

5. The immunoassay method of claim 1, wherein contact between the test sample and the second immunity substances is made by positioning a label phase partially up the immobilized phase by adding the liquid containing the second immunity substance partially up the immobilized phase and drying the liquid, the label phase comprising the second immunity substance in such manner that the second immunity substance can be released from the base material upon contact with water, allowing the test sample to be absorbed from one end of the water-absorbable base material, thereby to form a complex of said second immunity substance and the assay target substance, and binding said complex with the first immunity substance at the immobilized phase.

6. An immunoassay device comprising:

an immobilized phase comprising plural first immunity substances each specific for an assay target substance immobilized on a water-absorbable base material; and

a label phase comprising a labeled immunity substance comprising second immunity substances, said second immunity substances are labeled with colored latex particles which bind with one of said assay target substances in such a manner that the second immunity substance can be released from the base material upon contact with water, said immobilized phase comprising at least two different first immunity substances wherein said first immunity substances are specific for assay target substances selected from the group consisting of a combination of verotoxin-producing *Escherichia coli* and verotoxin, a combination of verotoxin and human hemoglobin, and a combination of verotoxin-producing *Escherichia coli* and human hemoglobin contained in a test sample, said first immunity substances being immobilized on different positions on the water-soluble base material.

7. An immunoassay kit comprising:

an immobilized phase comprising, on a water-absorbable base material, plural immobilized first immunity substances each specific for an assay target substance; and

a liquid containing second immunity substances, each of said second immunity substances is labeled with colored latex particles and is specific for one of said assay target substances, said assay target substances being at least two kinds of assay target substances selected from the group consisting of verotoxin-producing *Escherichia coli*, verotoxin and human hemoglobin, wherein the kit is specific for assay target substances selected from the group consisting of a combination of verotoxin-producing *Escherichia coli* and verotoxin, a combination of verotoxin and human hemoglobin, and a combination of verotoxin-producing *Escherichia coli* and human hemoglobin contained in a test sample.

IX. EVIDENCE APPENDIX

No evidence was submitted in this case under 37 C.F.R. 1.130, 1.131, or 1.132, and no evidence was entered separately by the Examiner.

X. RELATED PROCEEDINGS APPENDIX

No decisions have been rendered in any court or by the Board in a related appeal or interference.

Respectfully submitted,

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